ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49
55129 Mainz
Tel.: 06131-9258-0
Fax: 06131-9258-58

Thyroglobulin

ORG 5TG

96 Tests

Immunometric Enzyme Immunoassay for the quantitative determination of thyroglobulin (hTG) in serum or plasma

CE

Instruction for use

August 2005

CONTENTS

CONTENTS 2
NAME AND INTENDED USE 3
SUMMARY AND EXPLANATION OF THE TEST 3
PRINCIPLE OF THE TEST 3
WARNINGS AND PRECAUTIONS 4
CONTENTS OF THE KIT 4
STORAGE AND STABILITY 5
MATERIALS REQUIRED 5
SPECIMEN COLLECTION, STORAGE AND HANDLING 5
PROCEDURAL NOTES 6
PREPARATION OF REAGENTS 6
TEST PROCEDURE 6
INTERPRETATION OF RESULTS 8
PERFORMANCE CHARACTERISTICS 9
LIMITATIONS OF PROCEDURE 9
INTERFERING SUBSTANCES 9
REFERENCES 10
INCUBATION SCHEME 11

NAME AND INTENDED USE

Thyroglobulin is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of thyroglobulin in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of various thyroid diseases such as thyroiditis and hyperthyroidism.

SUMMARY AND EXPLANATION OF THE TEST

Thyroglobulin (hTG) is a multiple glycosylated, water soluble iodoprotein. The molecular weight of appox. 660.000 Dalton is shared by two identical subunits. Thyroglobulin is synthesized in the thyrocytes of the thyroid gland and secreted into the lumen of the thyroid follicles. Iodination of the proteins tyrosyl residues lead to the precursors of the thyroid hormones T3 and T4. Finally the free T3 and free T4 are liberated into circulation, together with small amounts of thyroglobulin. Like for T3 and T4, synthesis and secretion of thyroglobulin is controlled by TSH and TRH. Suppressive medication using the thyroid hormones also leads to lower thyroglobulin serum concentration.

Elevated thyroglobulin serum concentrations have been reported in various thyroid diseases, such as

- hyperthyroidism
- non-toxic goiter
- thyroiditis
- differenciated thyroid carcinoma

Determination of thyroglobulin is a special prognostic value in Graves` disease patients undergoing therapy. Highly elevated hTG values at the end of a thyrostatic therapy are indicative for an early recidivation, whereas for patients with continuous low thyroglobulin concentrations prognosis tends to continual recovery.

A main application for the thyroglobulin determination is the post surgical monitoring of patients with differentiated thyroid carcinoma. After thyroidectomy, combined with x-ray therapy to destroy remaining thyroid tissue, one can expect an intermediate peak followed by a fast decrease of circulating thyroglobulin concentrations below the detection limit. Each renewed increase of serum thyroglobulin is indicative for residual thyroid tissue, a local recidivation of metastases.

Due to its easy repeatability in the routine monitoring of thyroid carcinoma patients, the determination of thyroglobulin is a valueable non-invasive alternative and supplement to 131I-scintigraphy

PRINCIPLE OF THE TEST

Highly specific anti-human-thyroglobulin antibodies are bound to microwells. Thyroglobulin, if present in diluted serum or plasma, binds to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human-thyroglobulin antibodies immunologically detects the bound patient thyroglobulin forming a antibody/thyroglobulin/conjugate complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of thyroglobulin present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly specific anti-human-thyroglobulin antibodies (polyclonal, rabbit). Ready to use.
6 vials, 1.5 ml each	Thyroglobulin calibrators (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) containing: 0; 3; 10; 30; 100; 300 ng/ml. Ready to use.
2 vials, 1,5 ml each	Thyroglobulin Controls in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) elevated (1) and normal (2), for the respective concentrations see the enclosed package insert. Ready to use.
1 vial, 3 ml	Thyroglobulin recovery control in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) containing 50 ng/ml human thyroglobulin. Ready to use.
1 vial, 15 ml	Sample buffer (Tris, NaN_3 <0,1% (w/w)), yellow, Ready to use.
1 vial, 15 ml	Enzyme conjugate solution (PBS, PROCLIN 300 <0,5% (v/v)), (light red) containing polyclonal rabbit anti-human-thyroglobulin; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.

1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN_3 <0,1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

TEST PROCEDURE

Recovery Test

The presence of autoantibodies against thyroglobulin (anti-TG) can interfere with the determination of thyroglobulin in serum.

Six immunogenic epitopes are present on the surface of the thyroglobulin molecule to which antibodies with different binding characteristics can attach. Autoantibodies against these epitopes will cause false negative results by binding endogenous thyroglobulin. Therefore, it is necessary to prove the presence of autoantibodies in patient samples. This can be done either by direct quantitative measurement with an anti-TG test or indirectly by recovery experiments in combination with the quantitative thyroglobulin determination. For this determination, exogenous TG with a defined concentration is added to the patient sample. Recovery of the exogenous TG can be calculated and provides evidence as to the presence of autoantibodies. This measurement provides a correlation of recovery values to the concentration of autoantibodies present. However, neither the correct antibody content nor the exact thyroglobulin concentration in presence of antibodies can be calculated with this measurement.

For the recovery test, samples have to be determined twice, directly and spiked with thyroglobulin. The percentage recovery is calculated according to the formula below:

$$\%\,recovery = \frac{ng/ml\ hTG(with\ recovery\ control)}{ng/ml\ hTG(without\ recovery\ control) + 50\,ng/ml} \times 100$$

Note: the recovery control contains 50 ng/ml human thyroglobulin.

Recovery should be expected in the range of 80-120 %. If percentage of thyroglobulin recovery is below or above this range, thyroglobulin values for the respective patient sample should be excluded for further assessment.

Procedure

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and undiluted patient samples (P..) and for the recovery test spiked patient samples (R..) (see paragraph **Recovery Test**) in duplicates.

	1	2	3	4	5	6
A	**SA**	**SE**	**P1**	**P3**		
B	**SA**	**SE**	**P1**	**P3**		
C	**SB**	**SF**	**R1**	**R3**		
D	**SB**	**SF**	**R1**	**R3**		
E	**SC**	**C1**	**P2**	**...**		
F	**SC**	**C1**	**P2**	**...**		
G	**SD**	**C2**	**R2**	**...**		
H	**SD**	**C2**	**R2**	**...**		

SA - SF: standards A to F
P1, P2... patient sample 1, 2 ...
R1, R2... spiked patient sample 1, 2 ...
C1: positive control
C2: negative control

2. Pipet **50 µl** of calibrators, controls and patient samples into the wells.
3. Add 50 µl sample buffer to the calibrators, controls and patient samples (P1, P2. ..) resp. **50 µl thyroglobulin recovery control** to the patient wells (R1, R2, ..) into the wells.
4. Incubate for 60 minutes at room temperature (20 - 28 °C).
5. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
6. Dispense 100 µl of enzyme conjugate into each well.
7. Incubate for 60 minutes at room temperature.
8. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
9. Dispense **100 µl** of TMB substrate solution into each well.
10. Incubate for 15 minutes at room temperature.
11. Add **100 µl** of stop solution to each well of the modules and let it stand for 5 minutes.
12. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600 - 690 nm is recommended.

The developed color is stable for at least 30 minutes. Read optical densities during this time.

Automation

The ORGENTEC Thyroglobulin ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for elevated Control (1) and normal Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calculation of results

For the thyroglobulin test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example

The figures below show typical test results for Thyroglobulin. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators									
No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl.Conc.	CV %
STA	A 1/B 1	0.065	0.062	0.063	0.4	0.2	0.3	0	4
STB	C 1/D 1	0.117	0.111	0.114	2.8	2.5	2.6	3	4
STC	E 1/F 1	0.221	0.209	0.215	10	9	10	10	4
STD	G 1/H 1	0.421	0.451	0.436	29	32	31	30	5
STE	A 2/B 2	0.930	0.933	0.932	102	102	102	100	0
STF	C 2/D 2	1.759	1.780	1.769	298	304	301	300	1

Interpretation of results

In the literature cut-off values for serum thyroglobulin of around 60 ng/ml, with a median of 5 to 10 ng/ml are reported. In newborn babies as well as in pregnant woman of the 3rd trimester higher thyroglobulin concentrations may be detected. For compehensive interpretation of thyroglobulin concentrations knowledge of alimentary iodine supply is indispensable. In regions with endemic goiter hTG values tend to be higher.

Using the thyroglobulin test kit, in healthy blood donors a normal range has been established.

2 to 50 ng/ml thyroglobulin

In patients with total thyroidectomy no detectable thyroglobulin should be present after a x-ray therapy. Every increase of thyroglobulin to detectable serum concentrations is indicative for recidivation on thyroglobulin producing metastasis.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high thyroglobulin concentrations were diluted with sample buffer and assayed in the Thyroglobulin kit. The assay shows linearity over the full measuring range.

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay		
Sample No	Mean (ng/ml)	CV (%)
1	33	1.9
2	93	2.4
3	227	3.2

Inter-Assay		
Sample No	Mean (ng/ml)	CV (%)
1	31	1.7
2	88	1.7
3	212	1.1

Sensitivity

The lower detection limit for Thyroglobulin has been determined at 1,0 ng/ml.

Specificity

The antisera (polyclonal, rabbit) used for coating of the microplate and in the enzyme conjugate are highly specific for the human thyroglobulin molecule.

Calibration

The quantitative test system for Thyroglobulin is calibrated against the Certified Reference Material CRM 457 from BCR, Brussels for human Thyroglobulin.

LIMITATIONS OF PROCEDURE

The Thyroglobulin ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

REFERENCES

1. Marriq, S. et al. Polypeptide chains of 19-S thyroglobulin from several mammalian species and of porcine 27-S iodoprotein. Eur. J. Biochem. 1977; 79: 143 - 149.
2. Gärtner, D.F. et al. Evidence for autonomous thyroglobulin release from euthyroid and hyperthyroid nodular goiters - Thyroglobulin, a possible helpful parameter in diagnosis of non-malignant thyroid disorders. Klin. Wochenschr. 1983; 61: 737 - 741.
3. Gebel, F. et al. The site of leakage of intrafollicular thyroglobulin into the blood stream in simple human goiter. J. Clin. Endocrinol. Metab. 1983; 57: 915 - 919.
4. Uller, R.P. and van Herle, A.J. Effect of therapy on serum thyroglobulin levels in patients with Graves` disease. J. Clin. Endocrinol. Metab. 1978; 46: 747 - 755.
5. Gardner, et al. Serum thyroglobulin in normal subjects and patients with hyperthyroidism due to Graves` disease: effects of T3, iodine, 131J, and antithyroid drugs. Clin. Endocr. (Oxf.) 1979; 11: 585 - 594.
6. Kawamura, S. et al. Serum thyroglobulin changes in patients with Graves` disease treated with long term antithyroid drug therapy. J. Clin. Endocrinol. Metab. 1983; 56: 507 - 512.
7. Czernichow, P. et al. Plasma thyroglobulin measurements help determine the type of thyroid defect in congential hypothyrodism. J. Clin. Endocrinol. Metab. 1983; 56: 242 - 245.
8. Mariotti, S. et al. Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia.

   New Engl. J. Med. 1982; 307: 410 - 412.
9. Reiners, C. Klinische Wertigkeit der Thyreoglobulinbestimmung im Serum. Akt. Endokr. Stoffw. 1984; 5: 76 - 82.
10. Kastrup, J. et al. An enzyme linked immunosorbent assay for measurement of human serum thyroglobulin. Evaluation of the influence of thyroglobulin autoantibodies. Scand. J. Lab. Invest. 1985; 45: 471 - 476.
11. Feldt-Rasmussen, U. et al. Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vitro and in vivo. J. Endocrinol. Invest. 1985; 8: 571 - 576.
12. van Herle, A.J. and Uller, R.P Elevated serum thyroglobulin: a marker of metestases in differentiated thyroid carcinomas. J. Clin. Invest. 1975; 56: 272 - 277.

INCUBATION SCHEME

1. Pipet **100 µl** calibrator, control or patient sample

→ Incubate for **30 minutes** at room temperature

→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution

2. Pipet **100 µl** enzyme conjugate

→ Incubate for **15 minutes** at room temperature

→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution

3. Pipet **100 µl** substrate solution

→ Incubate for **15 minutes** at room temperature

4. Add **100 µl** stop solution

→ Leave untouched for **5 minutes**

→ Read at **450 nm**